Dissimilatory nitrate reductions in N_2O producing *Bacillus azotoformans* LMG 9581^T

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N_2O is a potent greenhouse gas and a contributor to ozone layer destructions. There are three N_2O producing microbial processes that have been studied thus far: denitrificaion, dissimilatory nitrate reduction to ammonium (DNRA), and nitrification. The former two respiratory pathways were previously thought to be mutually exclusive, but recently both pathways have been demonstrated to be functional in one organism *Shewanella loihica* PV-4. Soil bacteria *Bacillus azotoformans* LMG 9581^T also possesses these two pathways, besides, its genome analyses showed a remarkable redundancy of dissimilatory nitrogen reduction, with multiple copies of each denitrification gene as well as *nrf*AH, but has reduced capacity for nitrogen assimilation, with no *nas* operon nor *amtB* gene. We hypothesize that (i) NH_4^+ and NO_3^- could not assimilated, and (ii) DNRA, if expressed, would be used for energy conservation, not for nitrogen assimilation.

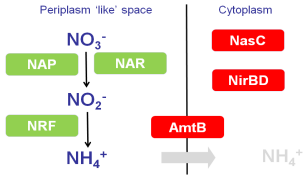

Fig 1. genes in green exist, genes in red are absent in the genome.

Topological model of enzymes involved in dissimilatory nitrate reduction (DNR) pathways

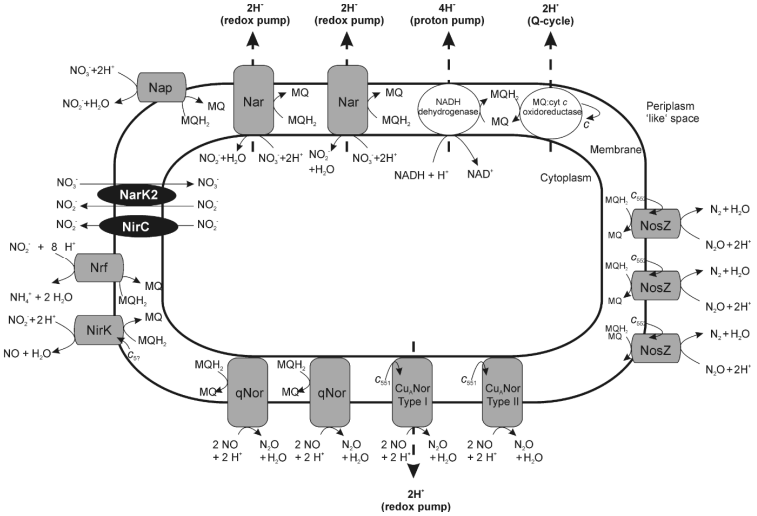

Fig 2. Electron transport chain of denitrification and DNRA, based on gene inventory of *B. azotoformans*.

Growth could be partially supported by NH4+ only if certain organic nitrogen exists

Table 1 . Growth observations of *B. azotoformans* after 15 days aerobic incubation under different combinations of nitrogen sources.

Nitrogen source	Growth[a]
4.6 mM NH4	-
100 mg/L Yeast extract , 4.6 mM NH4+	+
500 mg/L Yeast extract, 4.6 mM NH4+	+
1000mg/L Yeast extract, 4.6 mM NH4+	+
6.7 g/L YNB+[b]	+
6.7 g/L YNB-[c]	-
6.7 g/L YNB-, 10 mg/L L-histidine	-
6.7 g/L YNB-, 20 mg/L L-methionine	-
6.7 g/L YNB-, 20 mg/L L-tryptophan	-
YNB- supplemented with 5 g/L $(NH_4)_2SO4$	-
YNB- supplemented with 5 g/L $(NH_4)_2SO4$, 10 mg/L L-histidine	-
YNB- supplemented with 5 g/L $(NH_4)_2SO4$, 20 mg/L L-methionine	+
YNB- supplemented with 5 g/L $(NH_4)_2SO4$, 20 mg/L L-tryptophan	+
5 g/L $(NH_4)_2SO_4$	-
10 mg/L L-histidine, 20 mg/L L-methionine, 20 mg/L L-tryptophan	-
5 g/L $(NH_4)_2SO_4$, 10 mg/L L-histidine, 20 mg/L L-methionine, 20 mg/L L-tryptophan	+
114 mg/L casein enzymatic hydrolysate	+
1.14 g/L casein enzymatic hydrolysate	+
mixed vitamin B solutions	-
761 mg/L L-glutamate sodium or 658 mg/L L-glutamin with mixed vitamin B solutions	-

[a] '+' indicates growth observed ; '-' indicates no growth
[b] **'YNB+'** means defined yeast nitrogen base with 5g/l $(NH_4)_2SO_4$ with amino acids (10mg/l L-histidine, 20mg/l L-methionine, and 20mg/l L-tryptophan)
[c] **'YNB-'** means defined yeast nitrogen base without $(NH_4)_2SO_4$ without amino acids

A denitrification phenotype, no DNRA observed in Batch test

mineral media + 100mg/L yeast extract, 30 mM sodium acetate, 10 mM NO_3^-, 4.6 mM NH_4^+ if not indicated otherwise

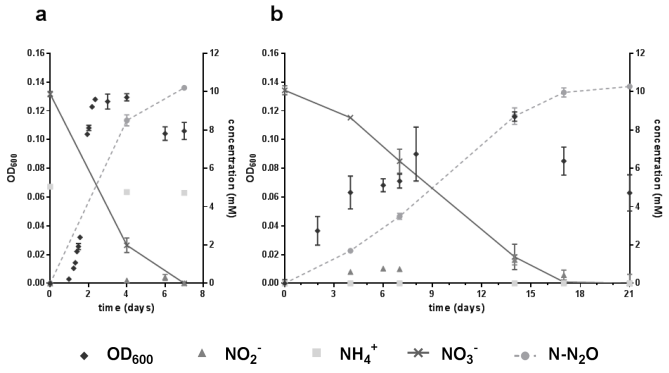

Fig 3. NH_4^+ concentration test: 4.6 mM (a), 1mM, 0.1mM and 0mM NH_4^+ (b). *n* > = 2. Similar growth is obtained in 4 different media (P >0.05), all initial NO_3^- converts to N_2O, growth rate is significantly different (P =2.9x10^{-5}, SPSS), no NH_4^+ production is observed.

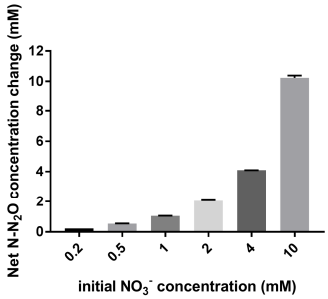

Figure 4. NO_3^- concentration test: 0.2 mM, 0.5 mM, 1 mM, 2 mM, 4 mM, 10 mM. *n* = 3. After growth, all initial NO_3^- converts to N_2O, no NH_4^+ production is observed. DNRA is not observed.

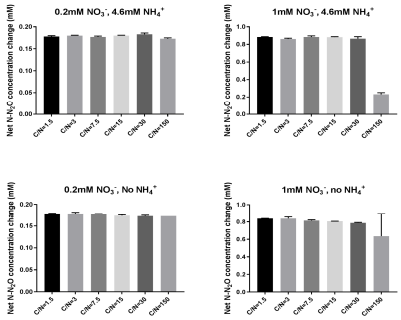

Figure 5. C/N-NO_3^- ratio test: C/N=1.5, 3, 7.5, 15, 30, 150, with 0.2 mM or 1 mM NO_3^-. *n* = 3. Slower growth was obtained after 3 days incubation under high C/N ratio of 150 (P <0.05, SPSS). After 7 days, all initial NO_3^- converts to N_2O. No NH_4^+ production is observed(P=0.308). DNRA is not observed .

Conclusions:

- ***B. azotoformans* has reduced N assimilation capacity, and requires organic nitrogen for assimilation.**
- **NH_4^+ can be assimilated only if certain organic nitrogen is supplied.**
- **NH_4^+ concentration has a clear influence on growth rate.**
- **Low NH_4^+ concentration, low NO_3^- concentration and high C/N ratio do not trigger DNRA in denitrifier *B. azotoformans* in batch test.**

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